Remarks

Claims 1, 7, 18, 19, 23 and 32 have been amended without any intention of disclaiming equivalents thereof. Upon entry of this paper, claims 1-10, 12-14, 16-25, 31-35, 126, 127 and 129-132 will be under consideration, and claims 11, 15, 26-30, and 128 have been withdrawn from consideration by the Examiner pursuant to his requirement for election of species.

Independent claim 1 has been amended to be limited to a method wherein the detection of the presence or absence of a post-translational modification of a fragment generated from the sample and bound to an immobilized capture agent is accomplished *using a secondary capture agent specific for said post-translational modification labeled with a detectable moiety.* Support for this amendment appears throughout the specification and claims as filed, for example, in claim 23 in the application as filed. Applicants have presented additional claim amendments for clarity and consistency. In addition, Applicants have amended paragraphs in the specification to remove possible embedded hyperlinks. Applicants believe that the aforementioned amendments introduce no new matter.

Response to Formal Objection

The specification presently stands as objected to because of embedded hyperlinks in certain paragraphs, for example, page 118, line 2 and page 121, line 18. Applicants thank Examiner Lin for his helpful discussion with Randall Morin, colleague of the undersigned, on September 21, 2006, to clarify this objection. Applicants have amended the specification to address this objection, and respectfully request that it be reconsidered and withdrawn.

Rejection under 35 USC § 112, Second Paragraph

All claims presently under consideration stand as rejected under 35 U.S.C. § 112, Second Paragraph. Claims 1, 18, and 19 have been amended to correct the indefiniteness deficiencies helpfully noted by the Examiner. This obviates the 35 USC § 112, Second Paragraph rejections. Accordingly, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Rejection Under 35 U.S.C. § 102(e)

Claims 1-3, 7-10, 12-14, 16-22, 31-35, 126, and 129 presently stand rejected under 35 U.S.C. § 102(e) as being anticipated by United States Patent Application Publication No. US 2002/0137119 A1 by Katz ("Katz"). Applicants respectfully request reconsideration and withdrawal of this rejection in view of the present amendments and following remarks.

All claims have been amended to be limited to a method wherein the detection of the presence or absence of a post-translational modification of a fragment generated from the sample and bound to an immobilized capture agent is accomplished *using a secondary capture agent specific for said post-translational modification labeled with a detectable moiety.* This is not disclosed or suggested by Katz, nor by any reference known to Applicants.

Furthermore, there are profound consequences inuring to the methods as now claimed by Applicants. Specifically, the claimed subject matter, for the first time as far as Applicants are aware, enables measurement of the state of *multiple* potential post translational modification (PTM) sites (of various kinds) of *multiple proteins*, with *positional detail* within a protein, and *in parallel.*

The latter feature is enabled by the selectivity provided by the protocol for digestion of the sample to produce peptides "comprising said potential post-translational modification site and a PET (proteome epitope tag) unique to said fragment within said sample" (see claim 1 part 2), in combination with the design of the immobilized capture agent which bind to those PETs. Thus, capture number one isolates specific protein fragments suspected to be modified (one or more), and captures them whether or not they are modified. Capture two identifies the type of modification, whether it is present, and optionally its extent. One is thus able to generate data sufficient to piece together the precise state of post-translational modification at multiple sites on multiple different proteins in parallel. This capability is very valuable and, as far as Applicants are aware, has not heretofore been achieved and is completely novel.

Accordingly, because Katz fails to teach or suggest at least these elements of claim 1 and claims 2-3, 7-10, 12-14, 16-22, 31-35, 126, and 129 by dependency, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Rejections Under 35 U.S.C. § 103(a) over Katz in view of Whaley

Claims 4-6 presently stand rejected under 35 U.S.C. § 103(a) as being obvious over Katz in view of Whaley *et al.* (1991) BIOLOGICAL MASS SPECTROPHOTOMETRY 20: 210-214 ("Whaley"). However, the combination of Katz and Whaley do not solve the deficiencies in the teachings and suggestions of Katz, as noted above. For example, Katz and Whaley, either alone or in combination, fail to teach or suggest a method, as in amended claim 1, that includes *using a secondary capture agent specific for said post-translational modification labeled with a detectable moiety.* Accordingly, because Katz and Whaley fail to teach or suggest at least this limitation of claim 1, and claims 4-6 by dependency, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Rejections Under 35 U.S.C. § 103(a) over Katz in view of Gembitsky

Claims 23-25, 127, and 130-132 presently stand rejected under 35 U.S.C. § 103(a) as being obvious over Katz in view of United States Patent Application Publication No. US 2005/0153298 by Gembitsky *et al.* ("Gembitsky"). Applicants respectfully request reconsideration and withdrawal of this rejection in view of the present amendments and following remarks.

First, neither Katz nor Gembitsky teach the following core features of the expressly claimed subject matter:

- Claim 1 part 2: "computationally identifying the amino acid sequence of one or more fragments . . . comprising said potential post-translational modification site and a PET (proteome epitope tag) unique to said fragment within said sample."
- Claim 1 part 5: "detecting, on said fragment bound to said capture agent [a PTM] . . . by using a secondary capture agent specific for said post-translational modification . . . ."

The teaching of Katz, in pertinent part with respect to the detection of PTM, is as follows:

> "The computer generated peptide products can also be analyzed according to the presence, absence and/or level of post-translational modifications. * * * Generally, preferred peptide products are those lacking any post-translational modification sites, since post-translationally modified amino acid sequences are often difficult to purify, and are frequently poor immunogens. * * * Notwithstanding from the above, peptide products which include post-translational modification, which

> indicate a biological activity of the polypeptide-of-interest can also be used by the present invention."

Thus, Katz teaches that capture agents can be generated so as to detect PTM protein fragments, but notes that such fragments "are frequently poor immunogens." He suggests no solution to this problem.

Furthermore, to be operative, the Katz strategy necessarily requires development of multiple antibodies that discriminate individual PTMs across the proteome in the sample. Given that sequence homology in different proteins in a proteome is common, this often is not possible. Thus, for example, an antibody that recognizes, say, Ser-Met-Arg-p-Tyr-His-Arg, as an epitope cannot discriminate among multiple different proteins displaying the same structure. This problem is not recognized in any of the applied references, much less solved or made obvious to the skilled artisan by their teachings.

Applicants solve this problem in two ways: 1) they need not make capture antibodies to the region of the PTM and hence can get round the issue of poor antigenicity as well as lack of sequence uniqueness, and 2) they use the sandwich assay such that the antibody to the PTM does not have to be unique to one PTM epitope on a single protein within the sample. In other words, an antibody or a dye that recognizes and binds to, for example, a phosphorylated tyrosine residue, with minimal or no regard for its adjacent amino acid structure, can be used to detect phosphorylation (or lack thereof) on multiple different proteins and on multiple different potential phosphorylation sites within a protein.

Gembitsky teaches an application of the well known sandwich assay format wherein an array of immobilized antibodies to proteins in a sample are used to capture the proteins, and then a second antibody specific to some PTM is used to detect the presence or absence of the PTM on the captured proteins. The only examples involve phosphorylation at Tyr residues using a p-Tyr detection antibody, although other types of PTMs are said to be detectable. There are many difficulties and challenges apparent from an understanding of the Gembitsky process, which he does not appear to recognize, much less solve.

Because many proteins have multiple pTyr (or other) modifications, the Gembitsky approach is *not specific to a given site*, and at best could indicate whether a *given protein* in a

sample is modified or not. Gembitsky apparently does not recognize this as an issue, and mentions no modification to correct this drawback. Applicants methods solve this problem.

Additionally, Gembitsky cannot detect modifications other than those accessible on a protein's surface. Buried modifications are invisible to Gembitsky, but revealed in Applicants' methods.

Gembitsky does not even recognize the serious problem of steric inhibition of the simultaneous binding of multiple binders to closely spaced modifications on proteins. This problem is inherently alleviated by Applicants' methods, with its digestion step in most instances serving to permit separation of closely spaced potential PTM sites.

Gembitsky fails to recognize that protein solutions often are dynamic, with epitopes appearing and disappearing as the proteins interact, such as by forming complexes, or as one cleaves another, and therefore that capture profiles are very hard to reproduce. This problem is solved by Applicants' methods as denaturation and subsequent digestion essentially eliminate or greatly reduce such complexities.

Gembitsky suggests the use of thousands of capture antibodies, yet these do not exist and Gembitsky fails to disclose how to solve the bottleneck inherent in the creation of these antibodies. As noted by Katz, "post-translationally modified amino acid sequences are often difficult to purify, and are frequently poor immunogens." In contrast, Applicants can raise antibodies to *synthesized peptides*, facilitating binder production without requiring isolation and purification of thousands of proteins. Furthermore, in many cases Applicants' methods can use the *same antibody multiple times* on the same array to detect modification on different proteins and at different locations within the structure of multiple individual proteins.

Nowhere does Gembitsky suggest digestion of the proteins in his sample for any purpose, or that the immobilized capture agents should be directed to peptides produced by a digestion. There is no reason apparent from the disclosures of either Gembitsky or Katz why the sandwich assay disclosed by Gembitsky should be combined with the Katz disclosure of digestion of sample for any purpose, and there is no appreciation in either reference of the power of such a combination of features.

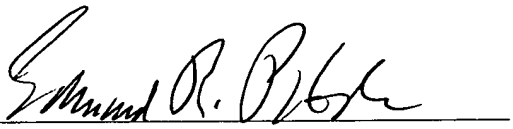
Again, the claimed subject matter of this application enables the measurement of the state of PTMs (of various kinds) of multiple proteins, in parallel, and *with positional detail within a protein.* By way of example, this invention permits determination of which of, say, five sites for modification, e.g., phosphorylation sites, in a given protein are in fact phosphorylated and which are not, and at the same time, to determine the extent and pattern of the same or a different class of PTM on multiple other proteins in the sample.

Accordingly, because neither Katz nor Gembitsky, alone or in combination, teach or suggest at least these elements of claim 1, and claims 23-25, 127, and 130-132 by dependency, Applicants respectfully request that this rejection be reconsidered and withdrawn.

Conclusion

Applicants believe that, in the view of the above amendments and responses, the pending claims are in condition for allowance. Early favorable action is respectfully solicited. The Office is invited to contact the undersigned with any questions about this submission.

Respectfully submitted,



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